Microvessel density (MVD) as a prognosticator in endometrial carcinoma

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Summary

Purpose: To assess microvessel density (MVD) as a marker for angiogenesis in endometrial carcinoma (EC) and normal endometrium at the proliferative and secretory phase, and to determine its prognostic value on survival among cases with EC.

Methods: Forty-three endometrial carcinoma cases were surgically staged and recruited for this case-control study. Tissue specimens from hysterectomies due to benign conditions (uterine descensus, myoma uteri, chronic pelvic pain, adenomyosis), that belonged to proliferative (n = 10) and secretory (n = 10) endometrium (n = 10), were studied as the control group (n = 20). MVD was assessed in hot areas where a high density of microvessels were detected within tumoral tissue and normal endometrium at proliferative and secretory phases. Among EC, various prognosticators such as tumor stage, histological and nuclear grade, tumor size, lympho-vascular space involvement (LVSI), cervical involvement, myometrial invasion, adnexal and lymph node involvement, peritoneal cytology and MVD were analysed in regard to survival.

Results: The mean age of cases with EC was 58.3 ± 1.4. MVD was apparently high in EC cases ($p < 0.05$). Among control cases, endometrium from proliferative and secretory phases of the menstrual cycle was not statistically different (48.5 ± 3.6 vs 47.4 ± 3.8, respectively). MVD was correlated with high surgical stage ($p < 0.001$), cervical involvement ($p = 0.01$), adnexal involvement ($p = 0.04$), lympho-vascular space involvement ($p = 0.02$), pelvic and para-aortic lymph node metastasis ($p < 0.001$) and positive peritoneal cytology ($p < 0.001$). On univariate analysis, with a MVD cut-off value of 81/0.739 mm^2, surgical stage ($p < 0.001$), LVSI ($p < 0.001$), retroperitoneal lymph node involvement ($p < 0.001$), adnexal metastasis ($p < 0.001$), peritoneal cytology ($p = 0.005$) and MVD count ($p < 0.001$) appeared to be independent factors for survival. On multivariate analysis, only pelvic lymph node involvement ($p = 0.03$) and MVD ($p = 0.02$) were found to be independent prognosticators on survival.

Conclusions: Angiogenesis is apparent in both initial and further evolution of a tumoral process. MVD appears to have a substantial prognostic value on survival in EC cases.

Key words: Microvessel density; Endometrial carcinoma; Prognosis.

Introduction

Angiogenesis is thought to be an important factor in tumor growth and metastatic spread. Microvessel density (MVD) within the primary tumor may provide useful prognostic information for several tumor types and has been considered to act as an independent variable to predict progression-free or overall survival and metastatic potential in endometrial carcinoma [1-3]. Several cellular interactions play a role in the progression and spread of endometrial carcinoma, among which, angiogenic factors and the receptor expressions, matrix degrading enzymes, tumor associated-macrophage systems, cyclooxygenases and anti-apoptotic factors can be elucidated [4-6].

In this study, our first objective was to assess MVD as a marker for angiogenesis, in endometrial carcinoma (EC), normal cyclic endometrium at both proliferative and secretory phases and as a second step, we aimed to determine the prognostic value of MVD count on survival among cases with EC.

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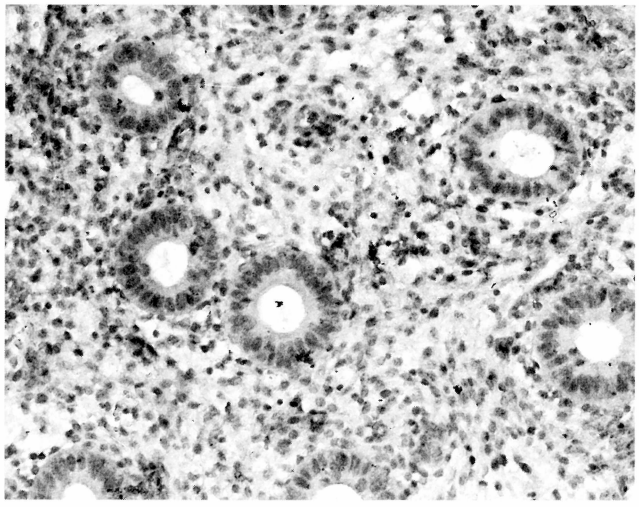
Materials and Methods

Clinical data and pathologic tissue samples of 43 endometrial carcinoma cases, from years 1990 to 1996, were obtained from the files at the University Hospital of Osmangazi University, Department of Obstetrics and Gynecology, and the Department of Pathology. Cases were surgically staged and recruited for this case-control study. Tissue specimens from hysterectomies due to benign conditions (uterine descensus, myoma uteri, chronic pelvic pain, adenomyosis), that belonged to the proliferative (n = 10) and secretory menstrual period (n = 10), were assigned as the control group (n = 20). All cases were staged surgically according to FIGO. Cases were evaluated in terms of surgical stage, tumor grade, tumor volume, lympho-vascular space involvement (LVSI), depth of stromal invasion, pelvic and paraaortic lymph node involvement. Tumor histology in all EC cases was endometrioid type endometrial carcinoma. Adjuvant radiation therapy was given in cases of moderately or poorly differentiated Stage Ib or Stage Ic-III disease. Radiation therapy consisted of brachytherapy and for higher stages, teletherapy, as a four-field-box external beam radiation.

Immunohistochemical method

Tissue sections were stained with FVIII monoclonal antibodies (Biogenex) via labelled streptavidin-biotin peroxidase (Zymed Universal Kit) immunohistochemical method. New paraffinized tissue blocks of 4 μ in thickness were prepared from all cases and then deparaffinized and rehydrated. Fol-

1a



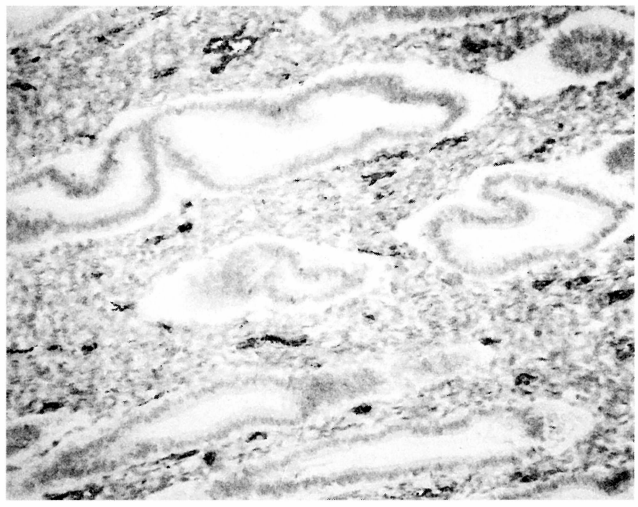


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Figure 1a. — Proliferative endometrium showing low microvessel density (immunoperoxidase stain for Factor VIII-related antigen; magnification x100).
Figure 1b. — Secretory endometrium showing low microvessel density (immunoperoxidase stain for Factor VIII-related antigen; magnification x100).

lowing the incubation period of one hour at 70°C in a microwave oven for 10 minutes, they were processed three times with 96% alcohol and xylol solution and finally, with distilled water. They were then treated with phosphate-buffered saline at pH 7.0 (Zymed, USA) for five minutes and dessicated.

Following this procedure, they were kept at room temperature for 10 minutes in hydrogen peroxide solution diluted with water. Upon treating with buffer solution, two drops of FVIII primary monoclonal antibodies were added onto tissue blocks that were kept for two hours at room temperature. Following the treatment with buffer solution, secondary monoclonal antibodies were added onto tissue blocks. After the treatment with buffer solution for five minutes, they were processed with 3 amino 9-ethylcarbazole (AEC substrate pack, Lab Vision Corporation), kept at room temperature for five minutes and then washed with distilled water. Counterstaining was done with hematoxylin.

Tissue blocks were mounted with slides (Lab Vision Ultramount). After scanning the immunostained section at low magnification (40x), the area of clear-cut cancer tissue with the greatest number of distinctly highlighted microvessels was selected. Any endothelial cell was regarded as a single countable microvessel, regardless of whether a lumen was visible or not in an examined area of 0.739 mm^2, acccording to guidelines published by Weidner *et al.* [8]. These positively stained areas were observed under a high magnified field x200 (x20 objective lens and x10 ocular lens) for MVD count. Regions where MVD had the highest value among three hot areas were considered for evaluation, shown in Figure 1a, Figure 1b and Figure 2. The final MVD value was determined by co-author pathologists (MA, UO) who were blinded to the clinical course of the patient.

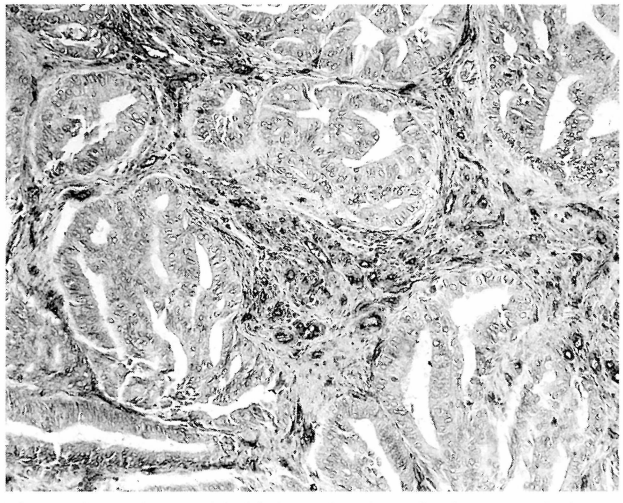

Figure 2. — Area with highest density of microvessels in an endometrial carcinoma (immunoperoxidase stain for Factor VIII-related antigen; magnification x100).

Statistical analyses were performed using the Statistical Package Program for Social Sciences, Computer Program (SPSS 10.0 Inc, Chicago, IL). The Student's *t*-test, Mann-Whitney U test and Krukal-Wallis analysis, when necessary, were used for evaluation of MVD count among normal and cancerous epithelia. A receiver operating curve (ROC) was constructed for determining different MVD count value cut-offs to predict the best survival. MVD value of 81 has been found to have the best sensitivity and specificity of 35.7% and 92.3%, respectively. MVD count changes among various prognosticators were evaluated by Fisher's exact χ^2 test. The Cox proportional-hazards model was used for univariate and multivariate analysis. Univariate analysis of overall survival was performed as outlined by Kaplan and Meier. Values were presented as mean ± standard error of mean (SEM). Statistical significance was set at a p value of < 0.05.

Results

The mean age of cases with EC was 58.3 ± 1.4. MVD was apparently high in EC cases ($p < 0.05$). Clinico-pathologic variables of EC cases are given in Table 1. Approximately 78% of EC cases showed one or more poor prognostic variables that necessitated adjuvant radiotherapy. With a cut-off value of MVD count as 81/0.739 mm^2 two-to-two contingency table analyses revealed that MVD count was correlated with advanced surgical stage ($p < 0.001$), cervical involvement ($p = 0.01$), adnexal involve-

Table 1. — *Clinico-pathologic variables of 43 endometrial carcinoma cases recruited in this study.*

Variables	n	%
Stage		
Ia	6	13.9
Ib	11	25.5
Ic	11	25.5
IIIa	1	2.3
IIIc	10	23.2
IVb	4	9.7
Grade		
I	15	34.9
II	23	53.5
III	5	11.6
Myometrial invasion		
No invasion	5	11.6
< 1/2 invasion	17	39.5
> 1/2 invasion	21	48.8
Cervical involvement		
+	13	30.2
–	30	69.8
LVSI		
+	3	7.0
–	40	93.0
Pelvic lymph node involvement		
+	11	25.6
–	32	79.1
Para-aortic lymph node involvement		
+	9	20.9
–	34	79.1
Tumor volume		
≤ 2 cm	9	20.9
> 2 cm	34	79.1
Peritoneal cytology		
+	6	13.9
–	37	86.1
Adnexal metastasis		
+	9	20.9
–	4	79.1
Adjuvant radiotherapy		
+	10	23.3
–	33	77.7

ment (p = 0.04), LVIS (p = 0.02), pelvic and para-aortic lymph node metastasis (p < 0.001) and positive peritoneal cytology (p < 0.001), as shown in Table 2. Tumor grade, tumor volume, myometrial invasion did not show any difference among MVD values of ≤ 81/0.739 mm² and > 81/0.739 mm², respectively. Furthermore, MVD count did not differ among the endometrium of proliferative (48.5 ± 3.6/0.739 mm²) and secretory phases of menstrual cycle (47.4 ± 3.8/0.739 mm²), as shown in Figure 3. However, among EC cases, MVD count (70.4 ± 26.5/0.739 mm²) was higher compared to both proliferative (p = 0.008) and secretory (p = 0.002) endometrium, respectively. On univariate analysis, with a MVD cut-off value of 81, surgical stage, lympho-vascular space metastasis, retroperitoneal lymph node involvement, adnexal involvement, peritoneal cytology and MVD appear to be independent factors for survival. On multivariate analysis, only pelvic lymph node involvement (p = 0.03) and MVD (p = 0.02) were found to be independent prognosticators on survival. Mean survival

Table 2. — *Distribution of poor prognostic variables among EC cases with MVD count ≤ or > 81/0.739 mm², respectively.*

	Microvessel count		
Variables	≤ 81	≥ 81	p value
Stage			
Ia, Ib, Ic	25	3	
IIIa or higher	4	11	< 0.001
Grade			
I and II	27	11	
III	2	3	0.055
Myometrial invasion			
< 1/2 invasion	18	4	
> 1/2 invasion	11	10	0.309
Cervical involvement			
+	24	6	
–	5	8	0.013
LVSI			
+	29	11	
–	0	3	0.029
Pelvic lymph node involvement			
+	27	5	
–	2	9	< 0.001
Para-aortic lymph node involvement			
+	28	6	
–	1	6	< 0.001
Tumor volume			
≤ 2 cm	7	2	
> 2 cm	22	12	0.693
Peritoneal cytology			
+	28	9	
–	0	6	0.002
Adnexal metastasis			
+	26	8	
–	3	6	0.040

times among cases with MVD < 81 and ≥ 81, were 61.1 ± 3.6 and 21.3 ± 4.8, respectively. Log-rank analysis for MVD to predict survival was assessed via Kaplan Meier survival curves, shown in Figure 4. As illustrated in the same figure, compared with cases of high MVD count, overall survival was higher in those cases with a low MVD count (p < 0.001).

Discussion

Current data pertaining to angiogenesis suggest that new vessel formation is pivotal for cyclic, regenerating endometrium and various endometrial pathologies such as endometrial hyperplasia and endometrial carcinoma [9, 10].

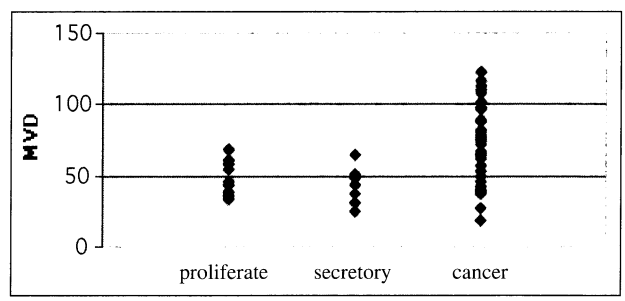

Figure 3. — MVD count in proliferative, secretory and cancerous epithelium.

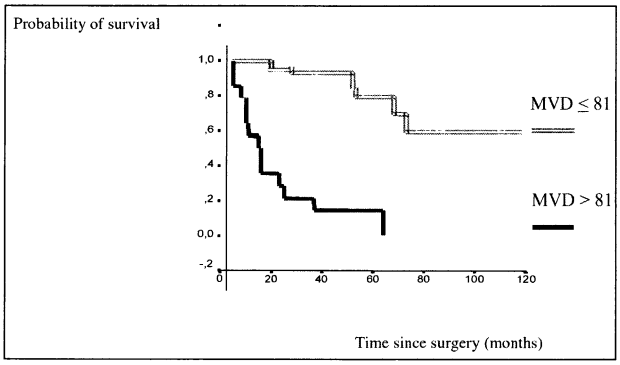

Figure 4. — Probability of overall survival in 43 patients with endometrial cancer with MVD at or below 81/0.739 mm² in the primary tumor (log-rank test, $p < 0.001$).

Table 3. — *Evaluation of various prognosticators deemed to affect survival via univariate and multivariate analysis.*

	UNIVARIATE			MULTIVARIATE		
Variable	B	B/SE	P	B	B/SE	P
Age	0.021	0.773	0.379	1.468	2.265	0.132
Stage	2.139	19.268	< 0.001*	0.862	0.126	0.722
Grade	0.529	2.539	0.111	-0.882	0.907	0.340
LVSI	3.216	12.324	< 0.001*	1.133	0.457	0.499
Pelvic LN meta	2.404	27.282	< 0.001*	3.976	4.392	0.036*
Para-aortic LN meta	3.223	21.767	< 0.001*	-0.149	0.013	0.908
Adnexal meta	1.502	9.743	0.001*	2.445	1.414	0.233
Peritoneal cytology	1.471	7.756	0.005*	0.823	0.612	0.432
Cervical meta	1.240	7.499	0.006*	0.138	0.020	0.885
Adj. Radiotherapy	-1.400	3.506	0.061	-0.923	0.354	0.551
MVD	2.624	23.324	< 0.001*	1.978	4.920	0.026*

*statistically significant; meta = metastasis.

Kirshner *et al.* [11], using immunohistochemical staining with factor VIII-related antigen, found an inverse relationship between tumor MVD and mean survival time in women with endometrial carcinoma. The present study also showed that microvessel count was an independent prognostic factor affecting survival. As shown by a Cox proportional-hazards model, surgical stage, LVSI, retroperitoneal lymph node involvement, adnexal involvement, peritoneal cytology and MVD were found to be independent and prognostically relevant factors. Obermeier *et al.* [12] demonstrated that high MVD count appeared to predict undifferentiated tumor, advanced stage, pelvic and paraaortic lymph node involvement. Furthermore, Salvesen *et al.* [13] suggested that with a lower MVD cut-off value of 68, cases with high MVD count had a lower 5-year survival compared to those with low MVD counts ($p = 0.004$). In our study, the best sensitivity and specificity were obtained with a MVD cut-off value of 81/0.739 mm².

Vascular endothelial growth factor, its receptor flt-1, KDR/flk-1 expressions were also suggested to be correlated with the stage of the endometrial carcinoma [14, 15]. In addition, hypoxic milieu of the tumor induces some regulatory proteins such as hypoxia-inducible factor 1 alpha (HIF-1alpha) and HIF-2alpha expression is associated with poor prognosis [16].

To conclude, high microvessel count has a poor survival of patients with endometrial carcinoma. Future studies are needed to clarify completely the association between the angiogenetic process and tumor development, aiding the physician to develop new therapeutic strategies.

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Prognostic factors in definitive radiotherapy of uterine cervical cancer

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Summary

Purpose: To determine the prognostic factors related to local control and survival in 257 patients with uterine cervical cancer treated with definitive radiotherapy (RT).

Materials and Methods: The medical records of 257 patients treated with definitive RT from January 1987 to December 1998 were reviewed retrospectively. Pretreatment and treatment parameters were analyzed to determine their prognostic value on local control and survival. Survival analyses were performed using the Kaplan-Meier method. The log-rank test was used for univariate analyses and the Cox regression model was used for multivariate analyses.

Results: Median age was 55 (range 25-82). Squamous cell carcinoma was the most common histologic type (89.1%). The distribution per FIGO Stage was IIA: 13.2%; IIB: 54.9%; IIIA: 3.9%; IIIB: 19.8%; IVA: 8.2%. Ninety-eight patients (38.1%) were treated with external RT alone; 134 (52.1%) received both external RT and intracavitary brachytherapy; 21 (8.2%) received external RT and chemotherapy and four (1.6%) received external RT, intracavitary brachytherapy and chemotherapy. Median follow-up duration was 50 months (range 24-155 months). The failure rate was 51.8% with 26.5% of patients having only local failure, 16.7% only distant failure and 8.6% both local and distant failure. Five-year local progression-free, disease-free and overall survival rates were 58.1%, 44% and 63.7%, respectively. In univariate analysis the prognostic factors identified for local progression-free survival were histology ($p = 0.008$), FIGO stage ($p < 0.001$), initial hemoglobin (Hgb) level ($p = 0.001$), total radiation dose ($p = 0.039$), use of brachytherapy ($p = 0.001$) and of chemotherapy ($p = 0.037$) and enlarged paraaortic nodes ($p = 0.016$). In multivariate analysis the prognostic factors were FIGO stage ($p = 0.014$), initial Hgb level ($p = 0.040$), and use of brachytherapy ($p = 0.013$). The prognostic factors identified for disease-free survival were histology ($p = 0.011$), FIGO stage ($p < 0.001$), initial Hgb level ($p < 0.001$), use of brachytherapy ($p = 0.001$) and of chemotherapy ($p = 0.014$) in univariate analysis; and FIGO stage ($p < 0.001$), initial Hgb level ($p = 0.017$), total tumor dose ($p = 0.034$), use of brachytherapy ($p = 0.006$) and of chemotherapy ($p = 0.021$) in multivariate analysis. Factors influencing overall survival were FIGO stage ($p < 0.001$), initial Hgb level ($p = 0.006$), overall treatment time ($p = 0.028$), total tumor dose ($p = 0.007$), use of brachytherapy ($p < 0.001$), enlarged paraaortic ($p < 0.001$) and pelvic nodes ($p = 0.004$) in univariate analysis; and FIGO stage ($p < 0.001$), overall treatment time ($p = 0.031$), enlarged paraaortic ($p = 0.007$) and pelvic lymph nodes ($p = 0.043$) in multivariate analysis.

Conclusion: Definitive RT is an effective treatment for patients with uterine cervical cancer. There are many prognostic factors influencing treatment outcome. Brachytherapy and chemotherapy must be added in appropriate patients to improve the outcome. Future prospective trials should be undertaken to confirm the validity of these factors and to individualize the treatment strategy for every patient.

Key words: Uterine cervical cancer; Prognostic factors; External radiotherapy; High-dose-rate brachytherapy.

Introduction

Carcinoma of the uterine cervix is the second most common malignant neoplasm in women worldwide [1]. The prognosis for this disease is markedly affected by the extent of disease at the time of diagnosis. Tumor control and survival in early stage lesions (Stages I and IIA) are comparable when patients are adequately treated with either definitive radiotherapy (RT) or radical surgery. Definitive radiotherapy has been the treatment of choice for locally advanced cervical cancer but with only little improvement in results over the last decades. Finally, recent results from each of five randomized phase III trials showed an overall survival advantage for cisplatin-based chemotherapy given concurrently with RT [2-6].

Several prognostic factors related to host characteristics (age, performance status, anemia), to tumor (stage, tumor size, histologic type, tumor grade, lymph node status) and to treatment factors (irradiation technique, irradiation dose, overall treatment time) have been identified that have a profound influence on outcome [1, 7-21].

This retrospective study evaluates the clinical- and treatment-related prognostic factors for local control and survival in patients with cervical cancer treated with definitive radiotherapy.

Materials and Methods

Between January 1987 and December 1998, 257 patients were treated with definitive radiotherapy for carcinoma of the uterine cervix at Ege University, Department of Radiation Oncology.

External RT was delivered by a Co 60 teletherapy machine prior to 1993 (40.9% of the patients) and by a 6-25 MV linear accelerator afterwards through individually shaped pelvic portals including the tumor and regional lymph nodes using

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AP/PA or a four-field technique (AP/PA and opposed laterals). The paraaortic field was added in the presence of enlarged paraaortic nodes. Inguinal lymphatics were included for patients with tumor extension beyond the middle third of the vagina. Daily fractionation dose was 1.8 Gy, 5 fractions per week. Total external RT dose was 64.8 Gy (with shrinking fields after 54 Gy) in patients who were not suitable for brachytherapy. Midline shielding was performed at 50.4 Gy in patients who were going to undergo intracavitary brachytherapy. Two fractions of 8.5 Gy (total dose: 17 Gy) with a one-week interval were applied to point A with the Rotterdam applicator via microSelectron high dose rate remote afterloader Ir-192. External irradiation was stopped at 54 Gy if there was no parametrial involvement. In cases of parametrial involvement 3 fractions of 1.8 Gy parametrial boost was added after 54 Gy.

Ninety-six-hour continuous infusion of 5-FU (1000 mg/m^2/day) was applied to 25 patients for the first and the last four days of external radiotherapy.

The patients were followed by physical, radiological and laboratory examinations at 3-month intervals during the first two years, at 6-month intervals up to the fifth year and annually thereafter unless disease progression necessitated shorter interval intervention.

Median follow-up duration was 50 months (range 24-155 months). Survival analyses were performed using the Kaplan-Meier method. Potential parameters analyzed to assess their impact on local control, disease-free and overall survival included patient age, FIGO stage, histologic type, initial Hgb level, overall treatment time, total tumor dose, addition of brachytherapy and/or chemotherapy to external RT and enlarged pelvic or paraaortic nodes. The log-rank test was used for univariate analyses and the Cox regression model for multivariate analyses.

Results

Median age of the patients was 55 (range 25-82). Squamous cell carcinomas represented 89.1% of tumors. In nine patients histological classification was not stated. Thirty-four patients (13.2%) had FIGO Stage IIA, 141 (54.9%) had Stage IIB, 10 (3.9%) had Stage IIIA, 51 (19.8%) had Stage IIIB and 21 (8.2%) had Stage IVA disease. Thirty-nine patients (15.2%) presented with enlarged pelvic or paraaortic nodes determined by ultrasound or computerized tomography. One-hundred and nineteen patients did not undergo intracavitary brachytherapy (72 of them due to lack of brachytherapy apparatus in our department prior to 1993 and 47 of them due to either advanced stage or insufficient response to external RT). Patient characteristics are indicated in Table 1.

The failure rate was 51.8% (133/257) with 26.5% (68/257) of patients having only local failure, 16.7% (43/257) having only distant failure and 8.6% (22/257) having both local and distant failure. Local-progression developed within a median of 17 months (4-120 months) and distant failure developed within a median of 13 months (1-120 months) after RT. Local failure rate by stages was 23.5%, 31.9%, 40.0%, 43.1% and 52.4% for Stages IIA, IIB, IIIA, IIIB and IVA, respectively. Distribution of distant metastasis by sites were: 44.6% lungs, 20% paraaortic lymph nodes, 9.2% bones, 7.7% liver, and the rest to other distant sites.

Five-year local progression-free, disease-free and overall survival rates were 58.1%, 44% and 63.7%, respectively. According to univariate analysis the prognostic factors identified for local progression-free survival were histology ($p = 0.008$), FIGO stage ($p < 0.001$), initial Hgb level ($p = 0.001$), total tumor dose ($p = 0.039$), use of brachytherapy ($p = 0.001$) and of chemotherapy ($p = 0.037$) and enlarged paraaortic nodes ($p = 0.016$), whereas in multivariate analysis the prognostic factors were stage ($p = 0.014$), initial Hgb level (0.040) and use of brachytherapy (0.013) (Table 2). For the whole group overall treatment time had no effect on local progression-free survival; however for Stage III and IV disease 5-year local progression-free survival rate was 54.7% when overall treatment time was shorter than 56 days (median) and 32.6% when it was longer than 56 days ($p = 0.016$). According to univariate analysis prognostic factors influencing disease-free survival were histology ($p = 0.011$), stage ($p < 0.001$), initial Hgb level ($p < 0.001$), use of brachytherapy ($p = 0.001$) and of chemotherapy ($p=0.014$) and enlarged paraaortic nodes ($p = 0.055$). In multivariate analysis advanced stage ($p < 0.001$), low Hgb level ($p = 0.017$), low total tumor dose ($p = 0.034$), ommision of brachytherapy ($p = 0.006$) and chemotherapy ($p = 0.021$) affected disease-free survival adversely (Table 3). According to univariate analysis prognostic factors identified for overall survival were stage ($p < 0.001$), initial Hgb level ($p = 0.006$), overall treatment time ($p = 0.028$), total tumor dose ($p = 0.007$), use of brachytherapy ($p < 0.001$), enlarged paraaortic ($p <0.001$) and pelvic lymph nodes ($p = 0.004$). In multivariate analysis these factors included stage ($p < 0.001$), overall treatment time ($p = 0.031$) and enlarged paraaortic ($p = 0.007$) and pelvic nodes ($p = 0.043$) (Table 4).

Table 1. — *Patient characteristics.*

	No. of patients	%
Age		
Median 55 (range 25-82)		
Histology		
Squamous cell carcinoma	229	89.1
Adenocarcinoma	19	7.4
Unspecified	9	3.5
FIGO Stage		
IIA	34	13.2
IIB	141	54.9
IIIA	10	3.9
IIIB	51	19.8
IVA	21	8.2
Enlarged pelvic lymph nodes		
No	195	75.9
Yes	32	12.5
Status unknown	30	11.6
Enlarged paraaortic lymph nodes		
No	223	86.8
Yes	7	2.7
Status unknown	27	10.5
Type of treatment		
ERT alone	98	38.1
ERT+BRT	134	52.1
ERT+CT	21	8.2
ERT+BRT+CT	4	1.6
Type of failure		
Local failure alone	68	26.5
Distant metastasis alone	43	16.7
Local + distant failure	22	8.6

ERT: External radiotherapy; BRT: Brachytherapy; CT: Chemotherapy

Discussion

The effectiveness of RT alone in the management of cervix carcinoma in all stages is well established [22, 23]. Although RT alone is often considered standard treatment for locally advanced cervix cancer the results continue to be relatively poor. Rates of local control vary from 60 to 85% in Stage IIb and from 50 to 60% in Stage III, pelvic failure alone being the first site of failure in approximately one-third of the patients. Overall incidence of pelvic recurrences for Stage IB, II, III and IVA disease were 12%, 20%, 41% and 72%, respectively in a series by Perez *et al.* including 1,499 patients [20]. Takeshi and associates [13] reported a 19.6% pelvic recurrence rate for 265 patients with Stage III disease treated by external radiotherapy and intracavitary brachytherapy. Montana [24] reported a 65% locoregional recurrence rate for Stage IIIA and 49% for Stage IIIB disease. Kim and Trotti [25] noted that pelvic failure rate varied from 4.6% to 8.2% in early stage disease (Stage IA, IB, IIA) and from 30.1% to 69.2% in advanced stages (Stage IIB, III, IVA). In the current study local failure rate was 33.5%, and according to the stages local failure rates were 23.5%, 31.9%, 40.0%, 43.1% and 52.4% for stages IIA, IIB, IIIA, IIIB and IVA, respectively.

Several prognostic factors have been identified that have a profound influence on outcome. Stage is clearly the most powerful determinant of outcome in virtually all studies; size and volume are also very important prognostic factors within each stage [7, 10, 13, 14]. Ten-year disease-free survival rates were reported as 82% for Stage I disease, 62% for Stage IIA and IIB and 36% for Stage III disease by Perez and associates [20]. Prempree *et al.* [10] showed a 53.2% 5-year disease-free survival rate in 94 patients with cervical cancer. In a retrospective analysis of 965 patients by Fyles *et al.* [7], FIGO stage was the most powerful prognostic factor followed by radiation dose and treatment duration. The Gynaecologic Oncology Group also found a significant correlation between progression-free interval and FIGO stage for Stages II, III and IV [14]. In our study FIGO stage was also the most powerful prognostic factor both in univariate and multivariate analyses for local control, disease-free and overall survival.

Conflicting results have been reported on the predictive value of histological subtype in cervical carcinoma. In some studies [7, 13, 26-28] the 5-year overall survival and relapse-free survival rates of patients with squamous cell carcinoma were significantly higher than those of the patients with adenocarcinoma while some studies were unable to establish a correlation [29, 30]. Adenocarcinoma histology was an adverse prognostic factor both for local control and disease-free survival in the present study. Five-year local progression-free and disease-free

Table 2. — *Prognostic factors for local progression-free survival.*

	5-year local recurrence-free survival (%)	Univariate analysis (p value)	Multivariate analysis (p value)
Age			
≤ 55	57.9	0.194	0.905
> 55	59.1		
Histology			
Squamous cell carcinoma	60.4	0.008	0.497
Adenocarcinoma	30.1		
Stage			
IIA-B	64.7	< 0.001	0.014
IIIA-B	47.7		
IVA	15.2*		
Hgb level (median)			
≤ 12.5 g/dl	44.8	0.001	0.040
> 12.5 g/dl	70.5		
Overall treatment time (median)			
≤ 56 days	62.1	0.085	0.433
> 56 days	55.5		
Total tumor dose (median)			
≤ 64.8 Gy	52.3	0.039	0.433
> 64.8 Gy	62.0		
Brachytherapy			
Yes	66.6	0.001	0.013
No	47.2		
Chemotherapy			
Yes	80.0	0.037	0.060
No	55.2		
Enlarged pelvic nodes			
Yes	52.6	0.110	0.197
No	61.9		
Enlarged paraaortic nodes			
Yes	42.8	0.016	0.095
No	60.6		

*Four-year local progression-free survival.

Table 3. — *Prognostic factors for disease-free survival.*

	5-year disease-free survival (%)	Univariate analysis (p value)	Multivariate analysis (p value)
Age			
≤ 55	43.7	0.325	0.594
> 55	44.7		
Histology			
Squamous cell carcinoma	46.5	0.011	0.106
Adenocarcinoma	20.4		
Stage			
IIA-B	50.9	< 0.001	< 0.001
IIIA-B	37.0		
IVA	6.9*		
Hgb level (median)			
≤ 12.5 g/dl	33.2	< 0.001	0.017
> 12.5 g/dl	57.2		
Overall treatment time (median)			
≤ 56 days	46.9	0.120	0.263
> 56 days	42.2		
Total tumor dose (median)			
≤ 64.8 Gy	41.0	0.154	0.034
> 64.8 Gy	50.8		
Brachytherapy			
Yes	51.0	0.001	0.006
No	35.8		
Chemotherapy			
Yes	72.1	0.014	0.021
No	40.7		
Enlarged pelvic nodes			
Yes	40.2	0.262	0.251
No	45.4		
Enlarged paraaortic nodes			
Yes	28.5	0.055	0.125
No	44.8		

*Four-year disease-free survival.

Table 4. — *Prognostic factors for overall survival.*

	5-year overall survival (%)	Univariate analysis (p value)	Multivariate analysis (p value)
Age			
≤ 55	61.8	0.507	0.605
> 55	65.8		
Histology			
Squamous cell carcinoma	63.7	0.577	0.452
Adenocarcinoma	56.6		
Stage			
IIA-B	75.1	< 0.001	< 0.001
IIIA-B	50.5		
IVA	0		
Hgb level (median)			
≤ 12.5 g/dl	52.4	0.006	0.151
> 12.5 g/dl	71.3		
Overall treatment time (median)			
≤ 56 days	67.9	0.028	0.031
> 56 days	61.2		
Total tumor dose (median)			
≤ 64.8 Gy	53.2	0.007	0.718
> 64.8 Gy	71.6		
Brachytherapy			
Yes	73.6	< 0.001	0.489
No	52.6		
Chemotherapy			
Yes	70.0	0.548	0.366
No	62.8		
Enlarged pelvic nodes			
Yes	48.8	0.004	0.043
No	65.7		
Enlarged paraaortic nodes			
Yes	28.5	< 0.001	0.007
No	63.8		

survival rates were 30.1% and 20.4%, respectively, for adenocarcinoma histology whereas the same rates were 60.4% and 46.5% for epidermoid carcinoma histology.

In Toita *et al's* study including 70 patients treated with radical irradiation pelvic nodal status had a significant predictive value on the incidence of distant metastases but not on pelvic control [21]. Fyles *et al.* stated that the presence of para-aortic nodal metastases was an independent prognostic factor, however positive lymph nodes in the pelvis only were not associated with an adverse outcome [7]. Stehman *et al.* analysed 626 patients with FIGO Stages I-IV who had surgical assessment of paraaortic nodes [14]. Paraaortic nodal involvement was associated with a 6-fold increase in risk for death and an 11-fold risk for disease progression and the status of the pelvic nodes was only important if the paraaortic lymph nodes were negative. Kapp *et al.* [30] showed in multivariate analysis that patients with enlarged pelvic and/or paraaortic nodes on pretreatment computerized tomography scans were predictive for disease-specific survival and distant metastases. In the current study the presence of pelvic and/or paraaortic lymphadenopathy was a significant prognostic factor for overall survival both in univariate and multivariate analyses.

Anemia during radiotherapy is shown to be an adverse prognostic factor for cervical carcinoma [31]. Severe anemia increases the hypoxic cell population thus producing progressive radioresistance which might adversely affect the therapeutic outcome. High Hgb levels showed a strong correlation with survival and locoregional control [7, 12, 13, 30]. Several cut-off values, pretreatment, treatment or post-treatment values have been used in studies, but the pretreatment value might most directly reflect the patient's condition. Girinsky *et al.* reported a higher rate of locoregional failure in patients whose Hgb level was lower than 10 g/dl [12]. Takeshi *et al.* [13] stated that progression-free and overall survival rates were lower in Stage III patients with Hgb levels lower than 9 g/dl. In a study by Fyles *et al.* transfusion during treatment was an adverse prognostic factor for survival and local control [7]. Kapp *et al.* [30] showed that low initial Hgb level was a powerful parameter for disease specific survival, disease-free survival and local control in 181 patients treated with external RT and HDR brachytherapy. In the current study pretreatment Hgb levels were detected in 229 patients and the median value was 12.5 g/dl. Hgb levels lower than the median value were an adverse prognostic factor both for local progression-free and disease-free survival in univariate and multivariate analyses.

Increasing radiation dose was correlated with improved survival and freedom from relapse [7, 20, 32]. In small tumors most doses are effective whereas in large tumors higher doses are required to be effective. The issue of dose response is somewhat controversial as the available data in the literature is derived from retrospective series rather than from randomized trials of different radiation doses. Similarly local control and overall survival were better in patients whose total tumor dose was higher than 64.8 Gy in our study although patients may have been selected for higher-dose treatment on the basis of performance status and extent of disease.

Several authors have described a worse outcome with prolongation of overall treatment time in patients treated with RT [7, 15-17, 19, 33-35]. This time effect is consistent with accelerated clonogen repopulation occurring during therapy, particularly in patients with advanced stage tumors, so treatment duration should be kept as short as possible. In an analysis of 837 patients Lanciano *et al.* [19] reported a highly significant decrease in pelvic tumor control and survival with prolongation of treatment times. Fyles *et al.* [16] demonstrated the adverse effect of increased treatment duration on pelvic tumor control in 830 patients with cervical carcinoma treated with irradiation alone and this effect was predominantly observed in Stages III and IV compared with Stages I and II. Girinsky *et al.* [17] also observed a loss of tumor control and overall survival when the treatment time exceeded 52 days in 386 patients with Stage IIB-III cervical carcinoma treated with RT. These three main studies concluded that the loss of local control and overall survival when treatment duration exceeds the expected threshold is about 1% per day and this effect is predominantly seen in Stage III. In the present study overall treatment time had no effect on local progression-free survival for Stage II; however for Stage III and IV disease 5-year local progression-free survival rate was 54.7% when overall treatment time was shorter than 56 days (median) and 32.6% when it was longer than 56

days (p = 0.016). Overall treatment time was a significant prognostic factor for overall survival both in univariate and multivariate analyses. These results suggest that unnecessary treatment delays should be avoided particularly in patients with Stage III and IV disease.

The curative potential of RT in the management of cervical carcinoma is greatly enhanced by the use of intracavitary brachytherapy. A very high dose of radiation to the central tumor can be delivered by brachytherapy while sparing to some degree the surrounding normal tissues. The American Brachytherapy Society recommends that brachytherapy must be included as a component of the definitive RT for cervical carcinoma based on the Patterns of Care studies that show that recurrences and complications are decreased when brachytherapy is used in addition to external RT [36]. In a retrospective study by Montana *et al.* [24] including 203 patients with Stage III cervical cancer it was found that addition of brachytherapy to external radiotherapy has an increased disease-free survival rate. Lanciano *et al.* reported an improvement in local control and survival rates with the addition of brachytherapy [32]. Perez *et al.* noted that it was the quality of brachytherapy application not the type and number which influences the pelvic recurrence rate [37]. Komaki *et al.* [38] demonstrated improved pelvic control with intracavitary brachytherapy for Stage III cervical carcinoma. Marcial *et al.* [39] stated that two or more intracavitary applications offered a higher paracentral point dose which appeared to be related to higher pelvic tumor control, lower distant metastatic rate and better survival. Logsdon *et al.* [40] showed that disease-specific survival of patients who had intracaviatry brachytherapy was nearly double that of patients who had only external RT in 983 Stage IIIb patients. Similarly in the current study local control and disease-free survival rates were better both in multivariate and univariate analysis in patients who underwent brachytherapy.

High rates of local control and survival can be achieved with definitive radiotherapy in early stage cervical carcinoma whereas treatment results are unsatisfactory for those with locally advanced disease. Many attempts have been made to improve upon these results including modifications in RT technique and combinations of RT with chemotherapy. Several studies were performed to test the effect of chemotherapy in locally advanced cervical cancer [41-44]. Hydroxyurea, mitomycin-C, 5-fluorouracil (5-FU) and cisplatin were the most commonly used cytotoxic agents. Finally, recent results from each of five randomized phase III trials showed an overall survival advantage for cisplatin-based chemotherapy given concurrently with RT [2-6]. The risk of death from cervical cancer was decreased by 30% to 50% by concurrent chemoradiation. In the present study 96-hour continuous infusion of 5-FU (1000 mg/m^2/day) was applied to 25 patients for the first and the last four days of external radiotherapy in 1990 for research. Although the number of patients was small the addition of chemotherapy was a significant prognostic factor for disease-free survival both in univariate and multivariate analyses. We started a new treatment protocol in January 2000 after the publication of the results of five randomized phase III trials. According to this protocol inoperable patients and operated patients carrying a high risk of locoregional failure are being treated by weekly cisplatin (40 mg/m^2) concurrent with radiotherapy.

In several studies it has been reported that distant metastasis was related to stage and was more frequent in patients with involved pelvic-paraaortic nodes and/or adenocarcinoma histology with the most frequent site being the lungs [14, 20, 21, 27, 45]. Distant metastasis rate increases to 56% in advanced stages [45]. In the current study distant metastasis rate was 25.3%, and the most frequent sites were the lungs and paraaortic nodes as stated in the literature.

Five-year overall survival rate varies from 31% to 67% in patients treated with definitive radiotherapy [7, 24, 43, 46]. Five-year overall survival rate was 63.7% in our study and the prognostic factors identified for overall survival were stage, initial Hgb level, overall treatment time, total tumor dose, use of brachytherapy, enlarged pelvic and paraaortic nodes in univariate analysis and stage, overall treatment time, and enlarged pelvic and paraaortic nodes in multivariate analysis.

Definitive RT is an effective treatment for patients with uterine cervical cancer. Based on the results of various studies brachytherapy and chemotherapy must be part of the treatment in appropriate patients to improve the outcome. There are many prognostic factors influencing treatment outcome. However future prospective trials should be undertaken to confirm the validity of these factors and to individualize the treatment strategy for every patient.

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